

Platinum® *Tfi* DNA Polymerase

Cat. no: 73684-050

Kit Size: 500 units

Conc. 5 U/μl

Store at -20°C (non-frost-free)

Description

Platinum® *Tfi* DNA Polymerase is recombinant *Tfi* DNA polymerase complexed with a proprietary antibody mix that inhibits polymerase activity at ambient temperature, allowing room-temperature reaction setup. Activity is restored after the initial denaturation step in PCR cycling at 94°C, providing an automatic “hot start” for increased specificity, sensitivity, and yield.

Tfi DNA Polymerase is purified from *E. coli* expressing cloned mutants of the *Thermus filiformis* DNA polymerase gene. This enzyme has both 5' → 3' polymerase and 5' → 3' exonuclease activity, but lacks 3' → 5' exonuclease activity. *Tfi* DNA polymerase is heat-stable and will synthesize DNA at elevated temperatures from single-stranded templates in the presence of a primer (Shandilya et al., 2004).

Platinum® *Tfi* DNA Polymerase can be used in protocols that currently use Platinum® *Taq* DNA Polymerase without modification. PCR performance is comparable to that of Platinum® *Taq* in yield, specificity, fidelity, and robustness. Like Platinum® *Taq*, Platinum® *Tfi* DNA Polymerase has a nontemplate-dependent terminal transferase activity that adds a single deoxyadenosine (A) to the 3' ends of PCR products.

Component

Amount

Platinum® *Tfi* DNA Polymerase
5X Platinum® *Tfi* Reaction Buffer
50 mM Magnesium Chloride

100 μl
4 × 1.3 ml
1 ml

Part no. 73684.pps

Rev. date: 30 June 2006

Guidelines for PCR

General PCR parameters and troubleshooting information are documented in Innis, et al (Innis et al., 1990). PCR reactions should be assembled in a DNA-free environment using clean, dedicated automatic pipettors and aerosol resistant barrier tips. Always keep the control DNA and other templates to be amplified isolated from the other components.

General Recommendations for PCR Optimization

The protocol on the following page provides general guidelines for PCR amplification. Optimal reaction conditions—including incubation times and temperatures, and amounts of polymerase, primers, MgCl₂, and template DNA—may vary.

- For genomic DNA, 1.0 unit of Platinum® *Tfi* DNA Polymerase is sufficient for amplifying most targets less than 1kb. Increasing the amount of enzyme to 2.0 units may improve yield.
- For plasmid DNA, 1.0 unit is optimal.
- A general scheme for PCR optimization should start with adjusting the annealing temperature. The optimal annealing temperature should be 5–10° lower than the T_m of the primers used. For higher specificity, it may be necessary to gradually increase the annealing temperature in steps of 2–3°C.

Unit Definition

One unit of Platinum® *Tfi* DNA Polymerase incorporates 10 nmol of deoxyribonucleotide into acid-precipitable material in 30 minutes at 74°C.

Basic PCR Protocol

Due to Platinum[®] *Tfi* DNA Polymerase's "hot-start" capability, the reaction can be set up at room temperature.

1. Program the thermal cycler as follows (note that the annealing temperature will vary depending on the T_m of your primers):

Initial denaturation: 94°C for 2 minutes

25–40 cycles of:

Denaturation: 94°C for 15–30 seconds

Annealing: T_m of primers minus 5–10°C for 30 seconds

Extension: 68°C for 1 minute per kb of PCR product

Final extension: 68°C for 10 minutes

2. Add the following components to a DNase/RNase-free microcentrifuge tube. For multiple reactions, prepare a master mix of common components to minimize reagent loss and enable accurate pipetting.

<u>Component</u>	<u>Volume</u>	<u>Final Concentration</u>
5X Platinum [®] <i>Tfi</i> Reaction Buffer	10 μ l	1X
10 mM dNTP mix, PCR grade	1 μ l	200 μ M each
50 mM MgCl ₂	1.5 μ l	1.5 mM
Primer mix (10 μ M each)	1 μ l	0.2 μ M each
Template DNA	\geq 1 μ l	as required
Platinum [®] <i>Tfi</i> DNA Polymerase	0.2–0.4 μ l	1–2 units*
Autoclaved distilled water	to 50 μ l	n/a

*May use up to 2 units for genomic DNA. See note on previous page.

3. Cap the tube, tap gently to mix, and centrifuge briefly to collect the contents.
4. Place the tube in the thermal cycler and run the program from Step 1. After cycling, maintain the reaction at 4°C. Samples can be stored at –20°C until use.
5. Analyze the amplification products by agarose gel electrophoresis. We recommend using E-Gel[®] 1.2% gels and TrackIt[™] 100 bp or 1kb Plus DNA ladders (see **Additional Products** on page 4).

Quality Control

Platinum[®] *Tfi* DNA Polymerase is evaluated in a DNA polymerization activity assay that measures the percent of polymerase inhibition versus an uninhibited control. Platinum[®] *Tfi* DNA Polymerase is also functionally tested for amplification and the absence of double- and single-stranded endonuclease activity, as well as the absence of contaminating exonuclease activity.

Additional Products

<u>Product</u>	<u>Amount</u>	<u>Catalog no.</u>
10 mM dNTP Mix, PCR Grade	100 µl	18427-013
10 mM dNTP Mix, PCR Grade	1 ml	18427-088
E-Gel [®] 1.2% Starter Pak	6 gels plus PowerBase [™]	G6000-01
E-Gel [®] 1.2% 18-Pak	18 gels	G5018-01
TrackIt [™] 100 bp DNA Ladder	100 applications	10488-058
TrackIt [™] 1kb Plus DNA Ladder	100 applications	10488-085

References

- Innis, M. A., Gelfand, D. H., Sninsky, J. J., and White, T. S. (eds) (1990) *PCR Protocols: A Guide to Methods and Applications*, Academic Press, San Diego, CA
- Shandilya, H., Griffiths, K., Flynn, E. K., Astatke, M., Shih, P. J., Lee, J. E., Gerard, G. F., Gibbs, M. D., and Bergquist, P. L. (2004) *Extremophiles* 8, 243-251

Limited Use Label License No. 5: Invitrogen Technology

The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of patents owned or controlled by Invitrogen Corporation which cover this product based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Licensing Department, Invitrogen Corporation, 1600 Faraday Avenue, Carlsbad, California 92008. Phone (760) 603-7200. Fax (760) 602-6500. Email: outlicensing@invitrogen.com

©2006 Invitrogen Corporation. All rights reserved.

For research use only. Not intended for any animal or human therapeutic or diagnostic use.